Foresight®

HEV IgM EIA Test Kit
Package Insert

REF I231-1211 | English

An enzyme immunoassay (EIA) for the qualitative detection of IgM antibodies to Hepatitis E Virus (HEV) in human serum or plasma.

For professional in vitro diagnostic use only.

INTENDED USE

The HEV IgM EIA Test Kit is an enzyme immunoassay for the qualitative detection of IgM antibodies to Hepatitis E Virus (HEV) in human serum or plasma. It is intended for screening and as an aid in the diagnosis of possible Hepatitis E Virus infection.

SUMMARY

Hepatitis E Virus is known as epidemic non-A, non-B hepatitis. Like Hepatitis A, it is an acute and short-lived illness that can sometimes cause liver failure. Infection with this virus was first documented in 1955 during an outbreak in New Delhi, India.[1] The viral particles are 27 to 34 nanometers in diameter, are non-enveloped and contain a single-strand of positive-sense RNA that is approximately 7300 bases in length. The virus particle was first visualized in 1983,[2] but was only molecularly cloned in 1990.[3]

The incidence of Hepatitis E is highest in adults between the ages of 15 and 40. Though children often contract this infection as well, they less frequently become symptomatic. For Hepatitis E is a "self-limiting" disease. It usually goes away by itself and the patient recovers. However, during several weeks of the infection, the disease severely impairs a person's ability to work, care for family members, and obtain food. Hepatitis E occasionally develops into an acute severe liver disease, and is fatal in about 2% of all cases.

The HEV IgM EIA Test Kit is an immunoassay for the qualitative detection of the presence of IgM antibodies to *Hepatitis E Virus* (HEV) in serum or plasma specimen. The test utilizes recombinant HEV antigens to selectively detect IgM antibodies to HEV in serum or plasma.

PRINCIPLE

The HEV IgM EIA Test Kit is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to HEV in human serum or plasma. The microwell plate is coated with anti-human IgM antibodies. During testing, the specimen diluent and the specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to HEV, it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-HEV IgM antibody complexes. If the specimens do not contain IgM antibodies to HEV, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated recombinant HEV antigens are added to the microwell plate and then incubated. The enzyme-conjugated recombinant HEV antigens will bind to the immobilized anti-human IgM antibody-HEV IgM antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of HEV IgM antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of HEV IgM antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450nm.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate, Calibrators and Controls. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The HEV IgM EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity	
			96 wells/kit	480 wells/kit
	HEV IgM Microwell Plate	Microwell plate coated with anti-human IgM antibodies	1 plate (96 wells/plate)	5 plates (96 wells/plate)
1	HEV IgM Conjugate	Recombinant HEV antigens bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 50 mL	5 x 50 mL
2A	Specimen Diluent	Tris buffer; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL
6	HEV IgM Negative Control	Diluted human serum non-reactive for HEV IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
7	HEV IgM Positive Control	Diluted human serum highly reactive for HEV IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
	Plate Sealers		3	15
	Package Insert		1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 15-30°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 10, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL for 96 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent) • Add 100 µL of Positive Control in wells D1 and E1. (Red Reagent)	• B1 and C1: Add 100 µL Negative Control • D1 and E1: Add 100 µL Positive Control
2	• Add 100 µL of Specimen Diluent to assigned wells starting at F1. (Green Reagent) • Add 10 µL of specimen to assigned wells starting at F1. Then a color change from green to blue will occur to verify that the specimen has been added.	• Starting F1: Add 100 µL Specimen Diluent • Starting F1: Add 10 µL specimen
3	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at room temperature (15-30°C) for 30 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at room temperature for 30 min
4	• Remove the Plate Sealer. • Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
5	• Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µL of Conjugate to each well except for the Blank well

6	• Cover the microplate plate with the Plate Sealer and incubate in a water bath or an incubator at room temperature (15-30°C) for 30 minutes ± 2 minutes.	• Cover the microwell plate with the Plate Sealer and incubate at room temperature for 30 min
7	• Repeat Step 4.	• Repeat Step 4
8	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
9	• Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at room temperature (15-30°C) for 15 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at room temperature for 15 min
10	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
11	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

CALCULATION OF RESULTS AND VALIDITY

1. Calculate the Mean Absorbance of Negative Control and Positive Control by referring to the table below.

Example of Negative Control Calculation

Item	Absorbance
Negative Control: Well B1	0.014
Negative Control: Well C1	0.012
Total Absorbance of Negative Control	0.014 + 0.012 = 0.026
Mean Absorbance of Negative Control	0.026/2 = 0.013
Blank Absorbance: Well A1	0.006
Mean Absorbance of Negative Control – Blank Absorbance	0.013- 0.006 = 0.007

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 0.500

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

3. Calculate the Cut-Off Value using the following formula if the test results are valid.
   Cut-Off value = NCx + 0.160
   (NCx = Mean Absorbance of Negative Control after subtraction of Blank Absorbance)

Example of Cut-Off Value Calculation

Item	Absorbance
Mean Absorbance of Negative Control – Blank Absorbance	0.007
Cut-Off Value	0.007 + 0.160 = 0.167

INTERPRETATION OF RESULTS

NON-REACTIVE: Specimens with absorbance less than the Cut-Off value are non-reactive for antibodies to HEV and may be considered negative.

REACTIVE:* Specimens with absorbance greater than or equal to the Cut-Off value are considered initially reactive for antibodies to HEV. The specimens should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the re-test are presumed to be repeatedly reactive and should be confirmed using confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

***NOTE**: Specimens with values within ±10% of the cut-off value should be retested in duplicates for final interpretation.

LIMITATIONS

1. The HEV IgM EIA Test Kit is used for the detection of IgM antibodies to HEV in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.
4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a Positive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The HEV IgM EIA Test Kit has been compared to a leading commercial HEV IgM EIA test using clinical specimens. The results show that the clinical sensitivity of the HEV IgM EIA Test Kit is 99.2%, and the clinical specificity is 99.6%.

HEV IgM EIA vs. Other EIA

Method		Other EIA		Total Results
	Results	Positive	Negative	
HEV IgM EIA	Positive	131	4	135
	Negative	1	963	964
Total Results		132	967	1099

Clinical Sensitivity: 99.2% (95.9-100.0%)* Clinical Specificity: 99.6% (99.0-99.9%)*
Overall Agreement: 99.6% (98.9-99.9%)* *95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 15 replicates of two specimens: a medium positive and a high positive.
Inter-Assay: Between-run precision has been determined by 3 independent assays on the same two specimens: a medium positive and a high positive. Three different lots of the HEV IgM EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance / Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	4.381	0.299	6.825	4.367	0.302	6.916
2	12.025	0.524	4.357	11.987	0.541	4.513

BIBLIOGRAPHY

1. Gupta DN, Smetana HF (1957). *"The histopathology of viral hepatitis as seen in the Delhi epidemic (1955-56)"*. Indian J. Med. Res. 45 (Suppl.): 101-13.
2. Balayan MS, Andjaparidze AG, Savinskaya SS, *et al* (1983). *"Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route"*. Intervirology 20 (1): 23-31.
3. Reyes GR, Purdy MA, Kim JP, *et al* (1990). *"Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis"*. Science 247 (4948): 1335-9.

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	EC REP	Authorized Representative
2°C 8°C	Store between 2-8°C	LOT	Lot Number	REF	Catalog #
Microwell Plate	Microwell Plate	HEV IgM	HEV IgM	Conjugate	Conjugate
Wash Buffer 25x	Wash Buffer (25x)	Substrate A	Substrate A	Substrate B	Substrate B
Specimen Diluent	Specimen Diluent	Stop Solution	Stop Solution	Control +	Positive Control
Control −	Negative Control	Plate Sealer	Plate Sealer	Package Insert	Package Insert

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